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Analogs of Pteroylglutamic Acid. VIII. 4-Alkoxy Derivatives

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As intermediates for a series of 4-alkoxypteridines, a new series of 2,4,5-triamino-6-alkoxypyrimidines was synthesized. These reacted with 1,2-dicarbonyl compounds to produce 4-alkoxypteridines having properties quite different from those of hitherto known pteridines. 4-Alkoxypteroylglutamic acids were not obtained in pure form because of the ease of hydrolysis of the 4-alkoxy groups, but a new synthesis of pteroylglutamic acid was achieved.

Replacement of the 4-hydroxyl group of pteroylglutamic acid (I)[1] and certain of its analogs[2] by a 4-amino group resulted in the formation of a number of substances which are powerful metabolite antagonists for I. These 4-amino analogs have produced temporary remissions in cases of acute leukemia in humans, and in leukemia and experimental tumors in animals.[3] An earlier paper from this laboratory[4] described the preparation of 4-alkylamino derivatives of pteroylglutamic acid, which were found to possess a much lower toxicity than that of the corresponding unsubstituted 4-amino analogs. Therefore, the importance of the substituent in the 4-position of the molecule is evident, and it was considered worthwhile to study the effect of other groups in the 4-position, particularly with the thought of obtaining compounds of lower toxicity.

In the present investigation the introduction of a 4-alkoxy group was attempted, and while 4-alkoxypteroylglutamic acids were not obtained in pure form due to the ease of hydrolysis of the 4-alkoxy group, a new synthesis of pteroylglutamic acid has been achieved by taking advantage of this property. In the case of the simpler pteridines, it has been possible to synthesize compounds such as 2-amino-4-methoxy-6,7-dimethylpteridine in pure form and to characterize them.

2,4-Diamino-6-chloropyrimidine[4] on treatment with sodium alkoxides yielded 2,4-diamino-6-alkoxypyrimidines. The 6-methoxy-, ethoxy-, butoxy- and benzyloxy- compounds were prepared, and it was found that these compounds could be nitrosated in the 5-position quite easily. Similarly, 2,4-diamino-6-benzylmercaptopyrimidine was prepared from the 6-chloro compound, and this also was nitrosated readily in the 5-position. Hitherto, nitrosation in the 5-position has been reported only for pyrimidines containing hydroxy or amino in the 4- and 6-positions where tautomerism is possible.[5] The 5-nitroso compounds were reduced with sodium dithionite or hydrogen sulfide, and the 2,4,5-triamino-6-alkoxypyrimidines resulted. 2,4,5-Triamino-6-methoxypyrimidine and the 6-ethoxy homolog were isolated as crystalline salts; in other cases it was more convenient to use the crude triamine, without isolation, in the synthesis of pteridines. On heating with alkali or acid the 6-alkoxy group was hydrolyzed to yield the corresponding 6-hydroxypyrimidines.

(1) C. W. Waller, *et al.*, THIS JOURNAL, **70**, 19 (1948); R. B. Angier, *et al.*, *Science*, **103**, 667 (1946).

(2) For the preceding paper in this series, see THIS JOURNAL, **73**, 2864 (1951).

(3) J. B. Thiersch and F. S. Philips, *Am. J. Med. Sciences*, **217**, 575 (1949).

(4) B. Roth, J. M. Smith, Jr., and M. E. Hultquist, THIS JOURNAL, **72**, 1914 (1950).

(5) B. Lythgoe, *et al.*, *J. Chem. Soc.*, 315 (1944).

2-Amino-4-alkoxy-6,7-dimethylpteridines were prepared by reaction of the 2,4,5-triamino-6-alkoxypyrimidines with diacetyl. These 4-alkoxypteridines have rather interesting properties as compared with previously described pteridines. The introduction of the alkoxy group resulted in substances with high solubility in the common organic solvents such as alcohol and acetone; furthermore, the ultraviolet absorption spectra were very different from those of the corresponding 4-hydroxy and 4-amino derivatives. This is to be expected since tautomerism is limited by the introduction of the alkoxy group. By treatment with weak alkali or acid the 4-alkoxypteridines were converted readily to the known 4-hydroxy compounds.

Since the conditions of the Waller reaction[1] are relatively mild, it was considered possible that 4-alkoxypteroylglutamic acids could be prepared by the reaction of 2,4,5-triamino-6-alkoxypyrimidines with 2,3-dibromopropanal and *p*-aminobenzoylglutamic acid in aqueous solution at *p*H 3 to 4. It is probable that some of the desired 4-alkoxypteroylglutamic acids were present in the crude products of the reaction, because comparison of the microbiological assay and chemical assays[6] indicated a growth activity approximately one-half that of pteroylglutamic acid of comparable purity. When attempts were made to purify the crude reaction product by the customary procedures the alkoxy group was lost and the only pure product isolated was pteroylglutamic acid, identified by its ultraviolet absorption spectra and by the microbiological assay with *Lactobacillus casei* and *Streptococcus faecalis* R. In an experiment in which a crude product resulting from 2,4,5-triamino-6-methoxypyrimidine was purified, samples were isolated and the methoxyl content, chemical assay, and microbiological assay were determined at each step in the purification. It was found that the methoxyl content decreased in proportion to the other two as the purity increased. Pteroylglutamic acid was obtained showing a chemical assay of 93.6%, microbiological assay of 92.1%, and a methoxyl content of 0.00% (see Table I).

Experimental

2,4-Diamino-6-methoxypyrimidine.—A mixture of 50 g. (0.35 mole) of 2,4-diamino-6-chloropyrimidine[4] and 20.5 g. (0.38 mole) of sodium methylate dissolved in 500 ml. of anhydrous methanol was heated for six hours in a steel autoclave at 120°. The mixture was cooled, filtered from sodium chloride and distilled almost to dryness. A tan solid crystallized; dry weight, 46 g. This was purified by repeated recrystallization from absolute alcohol, yielding a white product melting at 161–162°.

Anal. Calcd. for $C_5H_8N_4O$: C, 42.9; H, 5.72; N, 40.0. Found: C, 42.9; H, 5.94; N, 40.4.

(6) B. L. Hutchings, *et al.*, *J. Biol. Chem.*, **163**, 705 (1947).

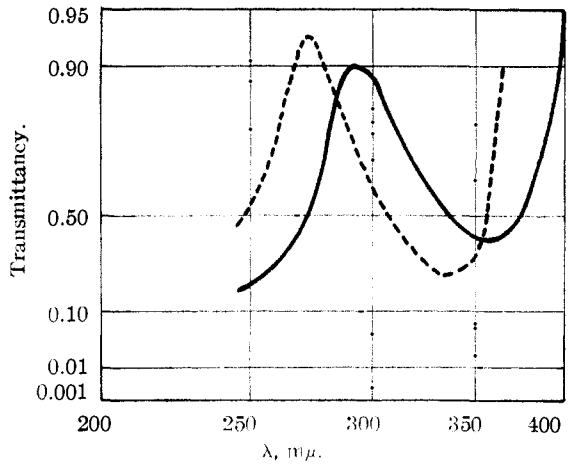

Fig. 1.—Ultraviolet absorption spectra of 2-amino-4-methoxy-6,7-dimethylpteridine (10 mg./l.) in 0.1 *N* sodium hydroxide solution (——) and in 0.1 *N* hydrochloric acid solution (- - - - -).

2,4-Diamino-6-ethoxypyrimidine.—To a solution of 24.4 g. (87.4% real, 0.381 mole) potassium hydroxide pellets in 500 ml. of absolute alcohol was added 50 g. (0.346 mole) of 2,4-diamino-6-chloropyrimidine. The mixture was refluxed with stirring for twenty hours, filtered from inorganic salts, and the filtrate cooled for several hours. A heavy white precipitate formed, which after filtering and drying weighed 28.5 g. This, upon recrystallization from alcohol, melted at 167–169°.

Anal. Calcd. for $C_6H_{10}N_4O$: C, 46.7; H, 6.50; N, 36.4. Found: C, 46.9; H, 6.62; N, 36.5.

2,4-Diamino-6-*n*-butoxypyrimidine.—To a solution of sodium *n*-butylate prepared from 1.75 g. (0.076 mole) of sodium in 100 ml. of *n*-butanol was added 10 g. (0.069 mole) of 2,4-diamino-6-chloropyrimidine. The mixture was heated to refluxing for four hours, filtered from sodium chloride and the butanol was removed by vacuum distillation. The residue solidified to a hard gum. A portion was recrystallized twice from Varnish Makers and Painters Naphtha, b.r. about 110–145°, yielding white crystals melting at 73–74°.

Anal. Calcd. for $C_8H_{14}N_4O$: C, 52.7; H, 7.75; N, 30.8. Found: C, 52.7; H, 7.98; N, 30.5.

2,4-Diamino-6-benzyloxypyrimidine.—To a solution of 3.6 g. of sodium in 100 ml. of benzyl alcohol was added 21.6 g. of 2,4-diamino-6-chloropyrimidine. The mixture was heated to 150–160° for three hours, after which the excess benzyl alcohol was distilled off. The oily residue was washed with water, leaving a gummy solid, which was used directly in the synthesis of 2,4-diamino-5-nitroso-6-benzyloxypyrimidine (see below).

2,4-Diamino-6-benzylthiopyrimidine.—A mixture of 5 g. of 2,4-diamino-6-chloropyrimidine, 4.72 g. of benzylmercaptan, 1.53 g. of ground sodium hydroxide pellets, and 15 ml. of glycol was heated at 150° for two hours. At first, two layers were present, which gradually became homogeneous, and a precipitate of sodium chloride formed. After cooling, the mixture was poured into water, yielding a white precipitate. The *p*H was adjusted to neutrality, and the product was filtered off and dried; weight, 5.2 g. This was used directly in the next reaction without further purification.

2,4-Diamino-5-nitroso-6-methoxypyrimidine.—Forty-six grams of 2,4-diamino-6-methoxypyrimidine was dissolved in 350 ml. of warm water, and 10 ml. of glacial acetic acid added. The mixture was adjusted to *p*H 4 with sodium hydroxide and clarified. It was then heated to 80° and a solution of 19 g. of sodium nitrite in 60 ml. of water added dropwise until a permanent spot on starch-potassium iodide paper was reached. The reaction was not instantaneous, approximately one hour being required for the addition of the nitrite. A dark red precipitate formed, which was filtered off and washed well with water; dry weight, 29 g.

Anal. Calcd. for $C_5H_7N_5O_2$: C, 35.5; H, 4.17; N, 41.4. Found: C, 35.6; H, 4.15; N, 41.3.

2,4-Diamino-5-nitroso-6-ethoxypyrimidine.—This was obtained as shiny red crystals by the above-described procedure.

Anal. Calcd. for $C_6H_9N_5O_2$: C, 39.4; H, 4.91; N, 38.2. Found: C, 39.2; H, 5.04; N, 37.9.

2,4-Diamino-5-nitroso-6-*n*-butoxypyrimidine.—A sample of crude 2,4-diamino-6-*n*-butoxypyrimidine (8.5 g.) was nitrosated as described above. A lavender product was formed which weighed 8.2 g.; m.p. 216–217° (dec.).

Anal. Calcd. for $C_8H_{13}N_5O_2$: C, 45.5; H, 6.21; N, 33.2. Found: C, 45.4; H, 6.20; N, 33.2.

2,4-Diamino-5-nitroso-6-benzyloxypyrimidine.—The crude 2,4-diamino-6-benzyloxypyrimidine described above was dissolved in 300 ml. of warm 30% acetic acid, clarified to remove impurities, and heated to 80°. A concentrated sodium nitrite solution was then added dropwise until a permanent spot on starch iodide paper was obtained. A shiny purple precipitate was formed, which was filtered off after cooling, washed with water, and dried; weight, 31.8 g. This was purified by recrystallization from acetone, in which it formed a blue-green solution.

Anal. Calcd. for $C_{11}H_{11}N_5O_2$: C, 53.9; H, 4.49. Found: C, 54.6; H, 4.65.

2,4-Diamino-5-nitroso-6-benzylthiopyrimidine.—This nitrosation was carried out by the above-described procedure, yielding an immediate purple precipitate, which was filtered off, washed well with water and alcohol and dried.

Anal. Calcd. for $C_{11}H_{11}N_5OS$: C, 50.7; H, 4.22; N, 26.8; S, 12.3. Found: C, 50.7; H, 4.35; N, 26.7; S, 12.2.

2,4,5-Triamino-6-methoxypyrimidine Sulfate.—A suspension of 29 g. of 2,4-diamino-5-nitroso-6-methoxypyrimidine in 230 ml. of water was heated to 50°, and 70 g. of sodium dithionite slowly added. A dark yellow solution was formed, which was filtered from a little gelatinous material and acidified to *p*H 2 with 1:1 (by volume) sulfuric acid. The mixture was cooled to 5°, yielding a white precipitate which was filtered off after two hours, washed with cold water, and air dried overnight and then at 45° for six hours. The product weighed 45.9 g., corresponding to a quantitative yield. A sample was purified for analysis by reprecipitation from an alkaline solution.

Anal. Calcd. for $C_5H_9N_5O \cdot H_2SO_4 \cdot H_2O$: C, 22.2; H, 4.80; S, 11.8. Found: C, 22.3; H, 4.80; S, 11.9.

2,4,5-Triamino-6-ethoxypyrimidine Sulfite.—The reduction of the nitroso derivative was carried out in the same fashion as described for the 6-methoxy derivative and the product isolated as the sulfite salt. This was purified by recrystallization from water.

Anal. Calcd. for $C_6H_{11}N_5O \cdot H_2SO_3$: C, 28.7; H, 5.20; N, 27.9; S, 12.8. Found: C, 28.9; H, 4.69; N, 27.8; S, 12.6.

2,4,5-Triamino-6-benzyloxypyrimidine.—Two grams of 2,4-diamino-5-nitroso-6-benzyloxypyrimidine was mixed with 79 ml. of alcohol and warmed to about 50°. Hydrogen sulfide was then bubbled rapidly into this mixture until complete decolorization of the nitroso compound had taken place, which took about twenty minutes. A yellow precipitate was formed, which was filtered from the warm solution and discarded. The filtrate was warmed under vacuum until all the residual hydrogen sulfide was removed, and the resultant solution was then used as such in subsequent reactions.

2-Amino-4-methoxy-6,7-dimethylpteridine.—One gram of 2,4,5-triamino-6-methoxypyrimidine sulfate was mixed with 15 ml. of water and neutralized to *p*H 7 with ammonium hydroxide. The solution was heated to 80°, and 0.32 g. of diacetyl in 3 ml. of water added over five minutes. A cream colored precipitate soon formed. The mixture was heated for ten minutes longer and cooled to 5°. The product was collected, and washed with water and acetone; dry weight, 0.3 g. After recrystallizing from dimethylformamide, it melted at 255–257° (dec.). It was found to be soluble in hot water, hot acetone and hot alcohol also. The substance was readily soluble in acid, and dissolved in hot dilute sodium hydroxide but precipitated on cooling. On standing, it slowly redissolved.

Anal. Calcd. for $C_9H_{11}N_5O$: C, 52.7; H, 5.37; N, 34.2; methoxyl, 15.1. Found: C, 52.8; H, 5.36; N, 34.2; methoxyl, 15.1.

In 0.1 sodium hydroxide solution it showed a maximum

at 356 mμ and a minimum at 292 mμ. In 0.1 *N* hydrochloric acid, a maximum occurred at 334 mμ, and a minimum at 272.5 mμ.

2-Amino-4-benzyloxy-6,7-dimethylpteridine.—To the above-described solution of 2,4,5-triamino-6-benzyloxypyrimidine was added 0.7 g. of diacetyl. The solution was heated under reflux for one hour. On cooling, a yellow precipitate was formed, which was filtered off and dried; weight, 1.8 g. This was purified by several recrystallizations from alcohol and then melted at 237–238° (dec.). The alcoholic solutions showed a blue fluorescence when diluted with water.

Anal. Calcd. for $C_{15}H_{15}N_5O$: C, 64.1; H, 5.35; N, 24.9. Found: C, 64.2; H, 5.95; N, 24.8.

Pteroylglutamic Acid.—The crude product from the condensation of 54.2 g. of 2,4,5-triamino-6-methoxypyrimidine, 26.6 g. of *p*-aminobenzoylglutamic acid, and 43.2 g. of 2,3-dibromopropanal, under the conditions described by Waller, *et al.*,[1] and modified by Wright, *et al.*,[7] was purified as follows.

Fifty-nine grams of the crude product (Sample A, Table I) was dissolved in 10 l. of water at 80° by the addition of 115 ml. of 5 *N* sodium hydroxide solution. After fifteen minutes 16 g. of calcium chloride in 37 ml. of water was added, and the insoluble material was removed by filtration with the aid of diatomaceous earth (Hyflo Super-Cel). A 200-ml. sample was removed, neutralized to *p*H 3 at 80°, cooled and the precipitate filtered off and dried (Sample B, Table I). The remainder of the filtrate was adjusted to *p*H 10.9 with aqueous zinc chloride, clarified, and the filtrate acidified to *p*H 3 at 80° with hydrochloric acid. It was cooled and the precipitate was collected on the filter; a small portion was dried (Sample C, Table I). The remainder was dissolved in 7500 ml. of water at 80° with the minimum amount of sodium hydroxide. The *p*H was adjusted to 7 while cooling to 20°, the insoluble material removed by filtration, and the solution adjusted to *p*H 3 at 80°. After cooling, the precipitate was collected and a small portion was dried for analysis (Sample D, Table I). The remainder was mixed with 18 g. of lime in 6 l. of water at 80°. After fifteen minutes the slurry was filtered and the filtrate neutralized to *p*H 10.9 with aqueous zinc chloride. Insoluble matter was removed by filtration, and the filtrate was acidified to *p*H 3 at 80°, cooled and the precipitate filtered. A small sample was dried (Sample E, Table I). The remainder was treated with lime and zinc chloride again as above (Sample F, Table I). The product was purified through the magnesium salt (Sample G, Table I), recrystallized from hydrochloric acid (Sample H, Table I), and finally a second magnesium salt (Sample J, Table I) to give pteroylglutamic acid which was identified by ultraviolet absorption spectra and by microbiological assay.

TABLE I

PTEROYLGLUTAMIC ACID SYNTHESIS FROM 2,4,5-TRIAMINO-6-METHOXYPYRIMIDINE

Sample	Chemical assay,[6] %	Biological assay *vs. S. faecalis* R	Methoxyl content, %
A	16.9	9.6	6.00
B	35.6	26.97	2.39
C	39.0	30.3	1.87
D	41.4	32.5	1.72
E	52.7	44.3	1.27
F	57.8	51.1	0.96
G	71.7	75.0	0.65
H	89.0	87.0	0.01
J	93.6	92.1	0.00

(7) W. B. Wright, Jr., *et al.*, THIS JOURNAL, **71**, 3014 (1949).

Pteroylglutamic Acid from 2,4,5-Triamino-6-ethoxypyrimidine Sulfite.—This reaction was carried out in the same manner as described for the condensation using 2,4,5-triamino-6-methoxypyrimidine. Purification by a similar procedure yielded yellow crystalline pteroylglutamic acid with a chemical assay of 91.4% and a bioassay of 94.6% as a growth stimulant for *S. faecalis* R.

Pteroylglutamic Acid from 2,4,5-Triamino-6-benzyloxypyrimidine.—Fifteen grams of 2,4-diamino-5-nitroso-6-benzyloxypyrimidine was reduced as above described. The resultant alcoholic solution (450 ml.) was diluted with 450 ml. of water, and 8.14 g. of *p*-aminobenzoylglutamic acid added. The *p*H was adjusted to 3.0, and solutions of 13.2 g. 2,3-dibromopropanal in 14 ml. of glacial acetic acid and 3.04 g. sodium dichromate in 17 ml. of water were added dropwise and simultaneously over a twenty-minute period, while keeping the temperature at 45° and the *p*H at 3. Heating at 45° was continued thirty minutes longer, after which the mixture was cooled and the light brown precipitate filtered off. After drying, this weighed 22.1 g. and had a chemical assay of 15.7% calculated as 4-benzyloxypteroylglutamic acid, and a bioassay of 5.65%.

Purification was accomplished using the same procedure that was used with 4-ethoxypteroylglutamic acid. A bright yellow product was obtained with a chemical assay of 90.3% calculated as pteroylglutamic acid, and a bioassay of 85.1% as a growth stimulant for *S. faecalis* R.

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